

## 1     Claims

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3     1.    A protein interaction system comprising

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5           a plurality of bait fusion proteins, each  
6           fusion protein comprising (i) a first fragment  
7           of fluorescent protein, a first peptide of  
8           interest and a linker portion interposed  
9           between the first peptide and first fluorescent  
10          fragment; wherein the linker portions of each  
11          bait fusion protein are of different lengths,  
12          and the first peptide of interest of each bait  
13          fusion protein is identical to the first  
14          peptide of interest in each of the other bait  
15          fusion proteins,

16

17          and (ii) at least one prey fusion protein  
18          comprising a fragment of fluorescent protein  
19          complementary to said first fragment of  
20          fluorescent protein, a second peptide of  
21          interest and a second linker portion interposed  
22          between the complementary fragment and the  
23          second peptide;

24

25          wherein, on interaction of a first peptide of  
26          interest with a second peptide of interest,  
27          the fragments of the fluorescent protein  
28          functionally associate to promote  
29          fluorescence.

30

31          2.    The protein interaction system as claimed in  
32          claim 1 or claim 2 wherein the linker

1 portions comprise in the range 5 to 100  
2 amino acid residues.

3

4 3. The protein interaction system as claimed in  
5 claim 2 wherein at least one linker portion  
6 comprises at least 20 amino acids.

7

8 4. The protein interaction system according to  
9 any one of claims 1 to 3, wherein the  
10 fragments of fluorescent protein are  
11 generatable through the introduction of a  
12 split point between the amino acids at  
13 positions 157 and 158, or (in a second  
14 embodiment) between the amino acids at  
15 positions 172 and 173 of the humanised form  
16 of Green Fluorescent Protein (SEQ ID NO 2).

17

18 5. The protein interaction system as claimed in  
19 any one of the preceding claims, wherein the  
20 system comprises a plurality of prey fusion  
21 proteins.

22

23 6. The protein interaction system as claimed in  
24 claim 5 wherein the linker portions of at  
25 least two prey fusion proteins are of  
26 different lengths.

27

28 7. The protein interaction system as claimed in  
29 claim 5 or 6 wherein at least two of the  
30 second peptides of interest of the prey  
31 fusion proteins are provided by different

1 amino acid sequences.

2

3 8. The protein interaction system as claimed in  
4 any one of the preceding claims wherein the  
5 first peptide is linked to the N terminus of  
6 the first fragment of fluorescent protein.

7

8 9. The protein interaction system as claimed in  
9 any one of claims 1 to 7 wherein the first  
10 peptide is linked to the C terminus of the  
11 first fragment of fluorescent protein.

12

13 10. The protein interaction system as claimed in  
14 any one of the preceding claims wherein the  
15 second peptide is linked to the N terminus  
16 of the complementary fragment of fluorescent  
17 protein.

18

19 11. The protein interaction system as claimed in  
20 any one of claims 1 to 9 wherein the second  
21 peptide is linked to the C terminus of the  
22 complementary fragment of fluorescent  
23 protein.

24

25 12. The protein interaction system as claimed in  
26 any one of the preceding claims further  
27 comprising at least a third fusion protein  
28 comprising at least a third fragment of  
29 fluorescent protein complementary to a first  
30 and / or second complementary fragment of  
31 fluorescent protein;  
32 wherein said at least third fragment is

1 linked to at least a third peptide of  
2 interest and at least a third linker is  
3 interposed between the at least third  
4 fragment and at least third peptide of  
5 interest wherein the at least third fragment  
6 of fluorescent protein is capable of  
7 functional association with a first and / or  
8 complementary fragment of fluorescent  
9 protein such that on functional association  
10 of said fragments fluorescence is enabled  
11 and on interaction of the first, second and  
12 third peptides of interest the fragments  
13 functionally complement each other to  
14 promote fluorescence.

15

16 13. A protein interaction system as claimed in  
17 any one of the preceding claims wherein the  
18 system is a cell based system.

19

20 14. A library of nucleic acid constructs  
21 comprising a plurality of nucleic acid  
22 constructs, each construct encoding  
23 (i) a first fragment of fluorescent protein  
24 capable of functional association with a  
25 complementary fragment of fluorescent  
26 protein such that on functional association  
27 of said first and complementary fragments  
28 fluorescence is enabled,  
29 (ii) a peptide of interest and  
30 (iii) a linker portion interposed between  
31 the peptide and first fragment of  
32 fluorescent protein; wherein the peptide of

- 1 interest encoded by each nucleic acid  
2 construct is the same and the linker portion  
3 encoded by each construct is of a different  
4 length to the linker encoded by each other  
5 construct.  
6
- 7 15. The library according to claim 14, wherein  
8 the linker portions comprise in the range 5  
9 to 100 amino acid residues.  
10
- 11 16. The library as claimed in claim 14 or claim  
12 15 wherein at least one linker portion  
13 comprises at least 20 amino acids.  
14
- 15 17. The library according to any one of claims  
16 14 to 16, wherein the fragments of  
17 fluorescent protein are generatable through  
18 the introduction of a split point between  
19 the amino acids at positions 157 and 158, or  
20 (in a second embodiment) between the amino  
21 acids at positions 172 and 173 of the  
22 humanised form of Green Fluorescent Protein  
23 (SEQ ID NO 2).  
24
- 25 18. An expression vector comprising at least one  
26 of the plurality of nucleic acid constructs  
27 as defined in claims 14 to 17, wherein the  
28 at least one nucleic acid construct encodes  
29 a fusion protein having a linker of at least  
30 20 amino acids.  
31

- 1        19. An expression vector comprising a plurality  
2        of nucleic acid constructs as defined in any  
3        one of claims 14 to 17.  
4
- 5        20. The expression vector according to claim 19,  
6        wherein at least one nucleic acid construct  
7        encodes a fusion protein having a linker of  
8        at least 20 amino acids.  
9
- 10       21. A cell transformed with a vector as claimed  
11       in any one of claims 18 to 20.  
12
- 13       22. A cell comprising a protein interaction  
14       system as claimed in any one of claims 1 to  
15       13.  
16
- 17       23. The cell according to claim 22, wherein the  
18       cell is a cell according to claim 21.  
19
- 20       24. An assay method for monitoring peptide  
21       interaction comprising the steps of  
22       (i) providing the protein interaction system  
23       of any one of claims 1 to 13;  
24       (ii) allowing the bait fusion proteins to  
25       come into contact with the prey fusion  
26       protein(s); and  
27       (iii) measuring fluorescence produced by the  
28       interaction of a first and second peptide of  
29       interest causing fragments of the  
30       fluorescent protein to functionally  
31       interact.  
32

- 1        25. The assay method according to claim 24,  
2                wherein the assay is a cell-based assay.  
3
- 4        26. The assay method according to claim 25,  
5                wherein the cell based assay is performed  
6                using one or more cells according to claim  
7                22 or claim 23.  
8
- 9        27. The method according to claim 25 or claim  
10               26, wherein the assay further comprises the  
11               step of determining the subcellular location  
12               of the interaction of the first and second  
13               peptides of interest in the at least one  
14               cell.  
15
- 16       28. The method according to any one of claims 24  
17               to 27, wherein the assay further comprises  
18               the step of determining the length of the  
19               linker(s) of those fusion proteins which  
20               allow the first fragment and complementary  
21               fragment of the fluorescent protein to  
22               functionally complement each other and  
23               enable fluorescence to be detected on  
24               interaction of the first and second peptide  
25               of interest.  
26
- 27       29. The method according to any one of claims 24  
28               to 28, wherein the assay comprises the steps  
29               of:  
30               providing a putative interaction modulating  
31               agent;  
32               measuring the fluorescence produced in the

1 presence of said putative modulating agent;  
2 comparing the measured fluorescence in the  
3 presence of the putative modulating agent  
4 with the measured fluorescence in the  
5 absence of the putative modulating agent;  
6 wherein a decrease in detection of  
7 fluorescence in the presence of the putative  
8 modulating agent relative to in the absence  
9 of the putative modulating agent is  
10 indicative that the putative modulating  
11 agent prevents or is an inhibitor of peptide  
12 interaction; and wherein an increase in  
13 detection of fluorescence in the presence of  
14 the putative modulating agent relative to in  
15 the absence of the putative modulating agent  
16 is indicative that the putative modulating  
17 agent promotes or enhances peptide  
18 interaction.

19

20 30. A kit comprising a library of nucleic acid  
21 constructs according to any one of claims 14  
22 to 17 and means to express the constructs.

23

24 31. The kit according to claim 30 which further  
25 includes at least one second nucleic acid  
26 construct which encodes a complementary  
27 fragment of fluorescent protein, a second  
28 peptide of interest and a second linker  
29 portion interposed between the complementary  
30 fragment and the second peptide of interest.

31



1        32. The kit as claimed in claim 31 wherein the  
2        kit comprises a plurality of second nucleic  
3        acid constructs, wherein the second peptides  
4        of interest encoded by the plurality of  
5        second nucleic acid constructs are each of  
6        different amino acid sequence.  
7  
8  
9